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THERMAL COMPENSATION IN PROTEIN AND RNA SYNTHESIS DURING THE INTERMOLT CYCLE OF THE AMERICAN LOBSTER, *HOMARUS AMERICANUS*

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The success of a species depends upon its ability to survive, grow and reproduce in its natural environment. The growth pattern in decapod crustaceans is known as the intermolt cycle. The survival and effective competition of most poikilothermic animals is aided by the phenomenon of thermal acclimation, enabling the organism to achieve a relative constancy of metabolic function in an environment of fluctuating temperature. Numerous reviews have delineated the myriad physiological and biochemical alterations associated with both molting and temperature acclimation (Passano, 1960; Huggins and Munday, 1968; Yamaoka and Scheer, 1970; Hohnke and Scheer, 1970; Rao, 1967; Hazel and Prosser, 1974). Given the diverse and sometimes conflicting metabolic demands associated with the two processes, it would be expected that the molt cycle condition would affect the patterns of thermal acclimation in crustaceans. McWhinnie and O'Connor (1967) reported that intermolt crayfish adaptively increase their oxygen consumption in response to cold temperatures, while premolt animals do not compensate.

The purpose of the present study is to explore the effect of the internal physiological condition of the organism (molt cycle stage) upon the metabolic response to an imposed environmental stress (acclimation temperature). The rates of incorporation of precursors into protein and RNA were measured to provide both specific information on changes in two important pathways, and more general information on metabolic changes because of the central role of these pathways in the synthesis of new enzymes and cellular components. Since pyrimidine precursors may enter the nucleotide pool for subsequent incorporation into RNA through a salvage pathway (uridine → UMP → RNA) or through *de novo* synthesis (CO_2 → orotic acid → UMP → RNA), the activities of both pathways were monitored.

Materials and Methods

Animals

Lobsters were collected from Narragansett Bay, Rhode Island, between late May and early September. Only reproductively immature animals, with a carapace length of 55–70 mm, were used. Molt condition was determined by the pleopod setogenesis method of Aiken (1973). Lobsters were acclimated at

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least one month in Dayno recirculating temperature-controlled aquaria, and fed three times weekly on chopped fish and mussels.

In vitro *assays*

The rate of protein synthesis and the activity of the salvage pathway for pyrimidine biosynthesis were assessed by measuring *in vitro* the rate of incorporation of ^{3}H-leucine or ^{3}H-uridine into the acid-insoluble fraction. The activity of the *de novo* pyrimidine pathway was assessed in two steps: the rate of incorporation of $NaH^{14}CO_3$ into orotic acid, and the rate of incorporation of orotic-^{14}C-acid into the acid-insoluble fraction. In all experiments, minces of 250 mg of tissue were incubated in 10 ml of lobster physiological saline (Cole, 1941) with 20 mM glucose and 30 mM imidazole buffer (pH 7.6) and adjusted to a saturating concentration of radioactive precursor [50 mM in ^{3}H-leucine (5 μCi); 20 mM in ^{3}H-uridine (5 μCi); 1 mM in orotic-^{14}C-acid (1 μCi)]; or 15 mM in $NaH^{14}CO_3$ (30 μCi), and 10 mM in 6-azauridine which inhibits the further metabolism of orotic acid (Handschumacher and Pasternak, 1958). All tissues showed linear incorporation with time for at least two hours. Saturating concentrations of nonradioactive precursor were added to the incubation medium to minimize variations in uptake and incorporation of the radioisotope due to alterations in membrane permeability and endogeneous precursor pool size. The saturation levels were determined by increasing the concentration of nonradioactive precursor while maintaining a constant specific activity (μCi/mmole) until there was no further enhancement of the incorporation rate.

After two hours of incubation with shaking at the desired temperature, the reaction was terminated with 10 ml ice cold 1 N $HClO_4$. The acid-insoluble, lipid-free residue was prepared by homogenizing the incubation mixture and washing the precipitate four times in 10 ml of 0.5 N $HClO_4$, once in water, and successively in 10 ml each of ethanol, ethanol/ether (1:1), and ethyl ether. The residue was dried, weighed and solubilized with 0.5 ml of Soluene-100 (Packard Instrument Corp.). Scintillation fluid (Das, 1967) was added and the samples were counted on a Packard Tri-Carb Model 3033 or Beckman Model 150 liquid scintillation counter for the time required to give a standard deviation of no greater than 5% of the total activity. Counting efficiency was about 30% for tritium and 70% for carbon-14.

The orotic acid synthesized during incubation with $NaH^{14}CO_3$ was isolated by co-crystalization with carrier orotate (Smith *et al.*, 1973), and recrystalized to a constant specific activity. The crystals were dissolved in 0.25 N KOH, diluted with Aquasol LSC cocktail (Packard Instruments) and counted in a Beckman Model 150 liquid scintillation counter for the time required to give a maximum standard deviation of 1.5% of the total activity.

RNA content

The RNA content of the tissues was determined by the orcinal colorimetric method of Drury (1948) performed on the acidified supernatant resulting from a mild alkaline hydrolysis of the acid-insoluble, lipid-free residue (0.3 N KOH for 2 hours at 37° C). Between 98–99% of the ^{3}H-uridine contained in the acid-

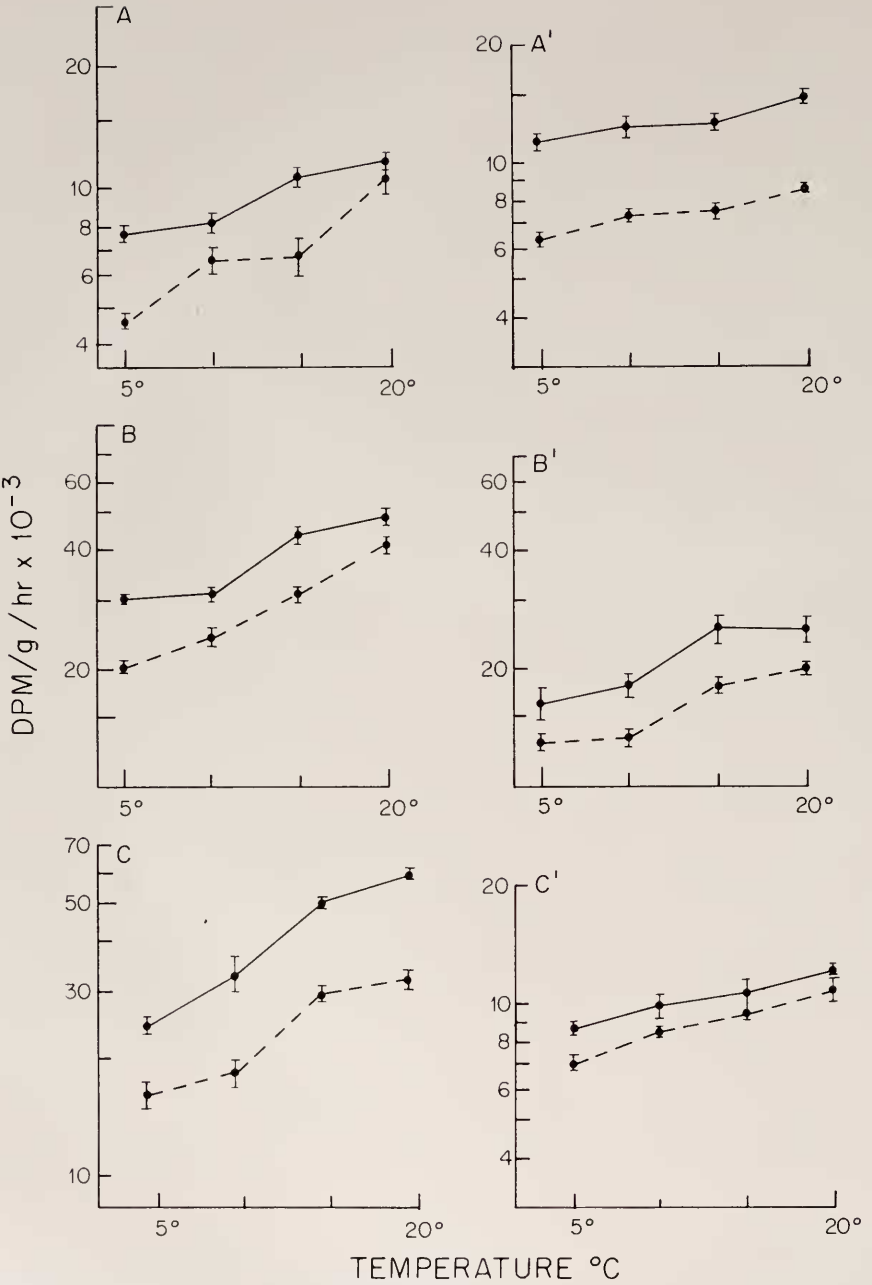

FIGURE 1. *Intermolt lobsters:* the effect of temperature acclimation on the rate of incorporation of ^{3}H–leucine (A, B, C) and ^{3}H-uridine (A′, B′, C′) into the acid insoluble fraction of midgut gland (A, A′), abdominal muscle (B, B′) and gill (C, C′). Solid lines are data from 5° C-acclimated lobsters, broken lines are data from 20° C-acclimated lobsters. Vertical

insoluble residue was rendered acid-soluble by mild alkaline hydrolysis, providing evidence that virtually all of the incorporation into nucleic acids is into RNA.

Results

The effect of the molt cycle stage upon the capacity for thermal acclimation in lobster tissues is shown in Figures 1 and 2. In these experiments, intermolt (stage C_4) (Fig. 1) and premolt (stage D_1'''-D_2) (Fig. 2) lobsters were acclimated to 5° C or 20° C for one month before tissues were assayed *in vitro* at a variety of temperatures. Temperature acclimation of intermolt lobsters results in compensatory shifts in the rates of incorporation of both ^{3}H-leucine and ^{3}H-uridine. In midgut gland, abdominal muscle, and gill tissue, the rate-versus-temperature (R-T) curve for tissue from cold-acclimated lobsters is translated to the left of that of warm-adapted animals.

In contrast, midgut gland and muscle from acclimated premolt lobsters (Figure 2) show no thermal compensation or an inverse compensation. Incorporation rates of warm-adapted tissue are either identical with or greater than incorporation rates of cold-adapted tissue.

Unlike the other premolt tissues, incorporation rates in gill exhibit a rotation, or change in the slope, of the R-T curves. Cold-acclimated curves are rotated counterclockwise with respect to leucine incorporation and slightly clockwise with respect to uridine incorporation.

The possibility of a difference in the rate of acclimation between intermolt and premolt animals was tested. Animals acclimated to one temperature regime were transferred to the other temperature; the rates of leucine and uridine incorporation were assayed every 3–4 days for a month. Most of the alterations in the elevation and slope of the R-T curves for the midgut gland and muscles of both intermolt and premolt lobsters were accomplished within ten days of transfer to the new temperature. The one exception was in the uridine incorporation rates in the intermolt midgut gland. The rates remained at warm-acclimated levels after ten days at 5° C, even though ten days was sufficient time for an adaptive response in the reciprocal acclimation direction (McCarthy, 1974).

The results of the studies on the activity of the *de novo* pathway for pyrimidine biosynthesis are shown in Table I. The rate of incorporation of $NaH^{14}CO_3$ into orotic acid in tissue of intermolt lobsters is relatively independent of the acclimation temperature. Alteration of the thermal regime has a more pronounced effect in tissues of premolt animals. Warm-acclimated rates are 25–30% higher than cold-adapted rates (inverse compensation). In 20° C-adapted lobsters, incorporation of $NaH^{14}CO_3$ into orotic acid in premolt tissue is 17–24% greater than in intermolt tissue.

The rate of incorporation of orotic-6-^{14}C acid into RNA is greater in cold-acclimated lobsters of either molt cycle condition, suggesting a compensatory acclimation to temperature. The activity of this portion of the pathway in the midgut gland of premolt lobsters is twice that of intermolt animals; activity is slightly

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bars indicate ± one standard error. Under experimental conditions, 1×10^3 DPM per gram tissue per hr is equivalent to the conversion of 45.0 nanomoles of ^{3}H-leucine into protein or 18.2 nanomoles of ^{3}H-uridine into RNA.

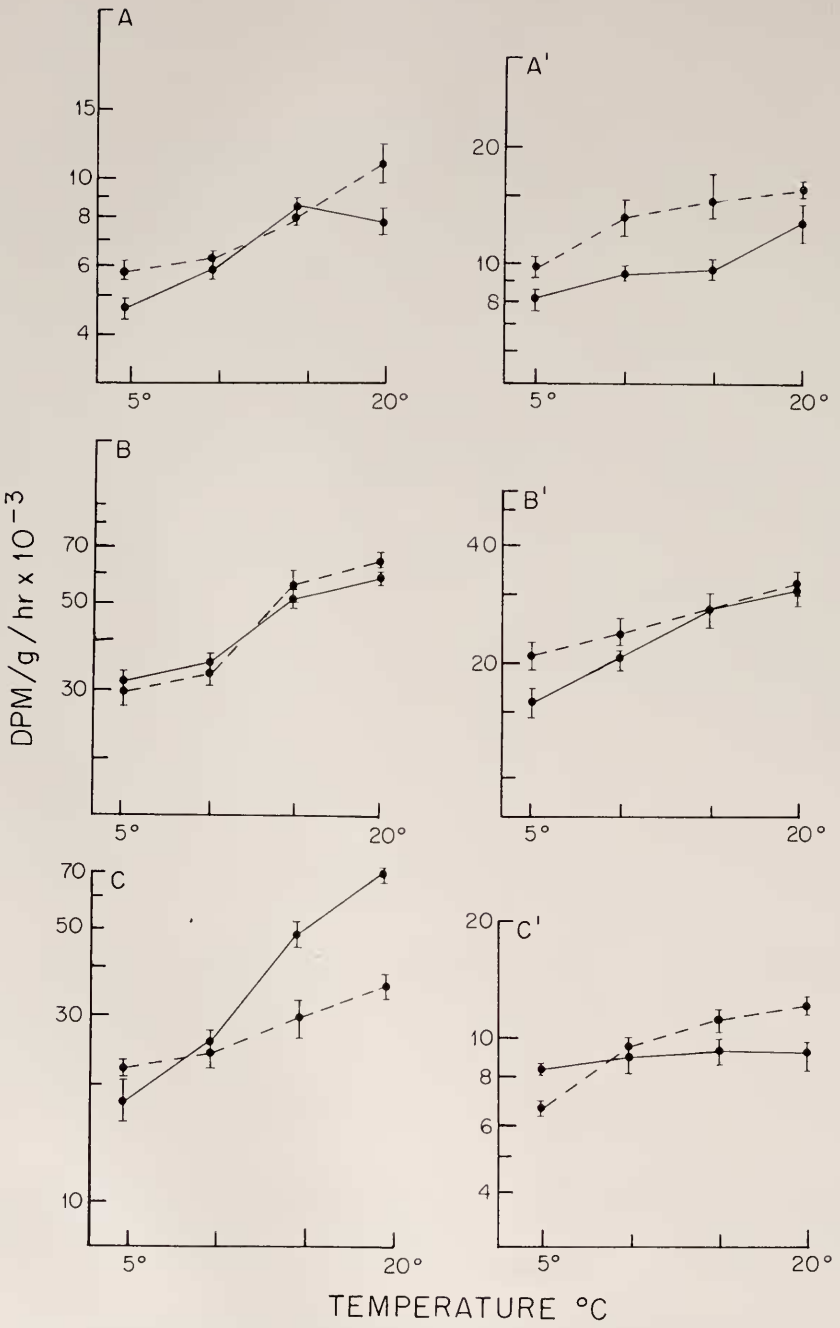

FIGURE 2. *Premolt lobsters:* the data are presented in the same way as in Figure 1.

greater in the muscle of intermolt as compared to premolt lobsters. There is a consistent decrease in the rate of orotate incorporation at increased incubation temperatures. The cause of this decrease is not immediately apparent; however, it is not due to a decreased rate of conversion of orotic acid to UMP. The rate of decarboxylation of orotic-2-^{14}C acid measured at 20° C was found to be 2–3 times greater than the rate at 5° C.

Both the molt cycle condition and temperature regime alter the concentration of RNA in the tissues (Table I). The RNA content of premolt mid-gut gland is 30–40% greater than that of intermolt tissue. The molt cycle had less effect upon the RNA content of the muscle and gill (McCarthy, 1974). Cold acclimation of both intermolt and premolt lobsters resulted in an increase in the RNA content of muscle and gill, but a decrease in hepatopancreas RNA.

Discussion

The response of an organism to an environmental stress such as temperature is clearly influenced by the internal physiological state of the animal. In this study, intermolt lobsters exhibited a positive acclimation response with respect to the *in vitro* rate of incorporation of precursors into protein and RNA. Lobsters in mid-premolt (stage D_1'''-D_2) showed either no acclimation or inverse acclimation, except in gill tissue. These results point out the need to consider the changing physiological conditions of an organism when evaluating its capacity to adapt to proposed environmental modifications.

The differential acclimation abilities of premolt and intermolt lobsters is also reflected in their capacity for resistance acclimations. Premolt lobsters acclimated to 15° C survive exposure to 28° C for a much shorter time than do intermolt animals with the same acclimation history. Resistance to low salinity also decreases in premolt lobsters (McLeese, 1956). The capacity of intermolt lobsters for temperature acclimation is undoubtedly of adaptive advantage in the natural environment. After summer molting, the lobsters continue to feed actively during the fall and early winter, even while temperatures are decreasing. Feeding activity (as estimated by stomach fullness) begins to increase from mid-winter, before water temperatures rise significantly (Ennis, 1973). The ability to metabolically compensate for the decreased environmental temperature permits the lobster to continue its active search for food. Because of this continued feeding activity, serum protein concentrations, which drop significantly following summer molting, recover to intermolt levels by mid-winter (Ennis, 1973).

While the acclimation capacity of intermolt lobsters is clearly of adaptive advantage, the absence of this temperature acclimation in the premolt animal is less readily understood. Yet this lack of an acclimation response may be of adaptive advantage, or at least impose no disadvantage, on the premolt lobster. Having ceased feeding near the beginning of the premolt state (Weiss, 1970), the lobster must complete preparations for the molt, including the synthesis of a new cuticle, using stored organic reserves. Metabolic compensation to temperature could compete with the molting process for the utilization of these reserves. In the fiddler crab, *Uca pugnax*, depletion of available organic reserves by starvation results in a loss of the ability to respond to cold exposure by a compensatory increase in metabolic rate (Vernberg, 1959). These observations suggest that there exists

TABLE I

Pyrimidine biosynthesis in the midgut gland and abdominal muscle of lobsters of different intermolt cycle and acclimation conditions. RNA is mg/g tissue, all others in nanomoles incorporated/g tissue/hr ± 1 standard error. Number of animals is in parentheses.

Molt stage	Acclimation temperature	$NaH^{14}CO_3$		Orotic-^{14}C-acid		^{3}H-uridine		RNA
		Incubation temperature						
		5° C	20° C	5° C	20° C	5° C	20° C	
Midgut gland Intermolt (stage C_4)	5° C	1.64 ±0.01 (3)	1.70 ±0.03 (3)	9.73 ±1.3 (3)	7.20 ±1.0 (3)	206.2 ±10.9 (12)	266.1 ±14.6 (10)	11.6 ±0.4 (10)
	20° C	1.64 ±0.05 (2)	1.97 ±0.06 (3)	8.03 ±1.0 (5)	4.40 ±0.4 (5)	115.6 ±5.5 (7)	157.5 ±3.6 (6)	14.4 ±0.3 (11)
Premolt (stage $D_1''' - D_2$)	5° C	1.59 ±0.04 (4)	1.46 ±0.03 (3)	20.84 ±0.8 (5)	17.65 ±2.0 (5)	148.3 ±9.1 (5)	236.0 ±27.3 (5)	18.6 ±0.6 (10)
	20° C	1.98 ±0.03 (4)	2.56 ±0.08 (2)	15.40 ±0.8 (4)	8.63 ±0.4 (3)	178.0 ±12.7 (6)	284.3 ±14.6 (7)	20.7 ±1.0 (6)
Muscle Intermolt (stage C_4)	5° C	1.63 ±0.02 (3)	1.56 ±0.01 (3)	13.86 ±1.0 (3)	10.01 ±1.2 (3)	292.6 ±25.5 (10)	466.5 ±38.2 (9)	0.92 ±0.05 (11)
	20° C	1.61 ±0.03 (3)	1.81 ±0.02 (3)	10.17 ±0.7 (5)	6.32 ±0.5 (5)	235.7 ±10.9 (7)	364.9 ±12.7 (6)	0.86 ±0.02 (8)
Premolt (stage $D_1''' - D_2$)	5° C	1.52 ±0.04 (4)	1.70 ±0.05 (4)	11.16 ±0.9 (5)	8.08 ±0.4 (5)	286.5 ±23.7 (6)	553.3 ±58.2 (6)	0.92 ±0.03 (8)
	20° C	2.12 ±0.04 (4)	2.33 ±0.01 (3)	9.62 ±1.1 (3)	6.65 ±0.7 (3)	376.7 ±32.8(5)	572.7 ±36.4 (6)	0.72 ±0.03 (8)

an integrative mechanism capable of preventing temperature acclimation when available organic reserves are required for what appear to be more vital metabolic needs. The compensatory mechanisms may also be of less value to the premolt lobster since in temperate latitudes, molting is a seasonal occurrence cued at least in part by an increased ambient temperature (Passano, 1960). Thus, the premolt animal should infrequently encounter the prolonged low temperatures which necessitate a compensatory metabolic reorganization.

The predominant pattern of acclimation in intermolt lobster tissue is a translation of the R-T curves. This is similar to the patterns exhibited by goldfish tissue with respect to leucine incorporation (Das and Prosser, 1967). It is also consistent with the mechanism for increased protein synthesis proposed by Haschemeyer (1968, 1969a, b), who found that the activity of elongation factor I in the protein synthetic pathway increased upon cold acclimation, resulting in a nonspecific increase in the rate of addition of aminoacyl residues to growing polypeptide chains. Such an increased rate of substrate addition would be expected to result in a translation, *i.e.*, a change in the elevation, but not the slope, of the R-T curves (Prosser and Brown, 1962). In this study, the cold-induced increase in the rate of leucine incorporation in intermolt midgut gland occurred several days before the corresponding increase in the rate of uridine incorporation or in the total RNA content of the tissue (McCarthy, 1974). This is also consistent with such a non-RNA mediated increase in the rate of protein synthesis.

While the molt cycle clearly affects the rates of incorporation of precursors into protein and RNA, the direction and magnitude of the change varies with the acclimation condition, incubation temperature, and tissue. Skinner (1965, 1968) and Gorell and Gilbert (1971) found an increase in both the level of leucine incorpora-

tion into proteins and the RNA content of the tissues of premolt animals and attempted to interpret the correlation as causative. The present study does not find a uniform increase in the rate of leucine incorporation. Yamaoka (1972) also found arginine incorporation in the crab, *Cancer magister,* to be greater in intermolt than premolt tissues. It seems likely, then, that control of the rate of protein synthesis during the molt cycle is not directly dependent upon the level of RNA in the tissues.

Although Skinner (1966) in *Gecarcinus lateralis* and Gorell and Gilbert (1971) in *Orconectes virilis* found RNA levels to increase in the premolt midgut gland, they reported a decrease and no change, respectively, in the rate of ^{3}H-uridine incorporation into RNA. The present study reports over a 50% increase in the rate of uridine incorporation in warm-adapted premolt midgut gland and abdominal muscle. In an effort to resolve this discrepancy, the possibility of competition between the salvage and *de novo* pathways for pyrimidine biosynthesis was examined. By comparison of premolt and intermolt rates measured in 20° C-acclimated lobsters assayed at 20° C (the more "normal" environmental conditions for premolt lobsters), it is clear that in both portions of the *de novo* pathway, as well as in the salvage pathway, there is an increase in amount of precursor (in nanomoles) incorporated into RNA in the premolt stage. This increased synthesis of pyrimidine nucleotides from both pathways is clearly the source of nucleotides for the increased level of RNA in premolt tissue.

The causes for the decrease in the conversion of orotate to RNA at increasing incubation temperatures is not fully elucidated. However, it is not due to a decreased rate of conversion of orotic acid to UMP, which was shown to increase at the higher incubation temperature. It is possible that the decreased incorporation into RNA is due to an increase in competition for nucleotides for purposes other than nucleic acid synthesis. The increased level of chitin biosynthesis beginning at stage D_1 may account for the increased competition for the pyrimidine nucleotides. The rate of ^{14}C-acetylglucosamine incorporation into both chitin and the activated intermediate, UDP-acetylglucosamine, increases by several orders of magnitude beginning at stage D_1 (Gwinn and Stevenson, 1973a, b). Although the nucleotide is recycled during the synthesis of the polysaccharide, the dramatically increased rate of chitin formation would presumably divert some of the pyrimidine nucleotides from nucleic acid metabolism.

Such an increase in competition for nucleotides would account for the decreased incorporation of ^{3}H-uridine into RNA found by the other workers. This effect would be minimized in the system used in this study, which measured incorporation in the presence of a saturating concentration of precursor. It should be noted that the amount of uridine incorporated into RNA (in nanomoles) by this system appears to be very much greater than that accounted for by increased competition for nucleotides at elevated incubation temperatures. It is unlikely, therefore, that the R-T curves for uridine incorporation are significantly influenced by this effect.

The incorporation of $NaH^{14}CO_3$ into orotic acid and orotic-^{14}C-acid into RNA reported here is, to our knowledge, the first report which demonstrates the existence of the complete *de novo* pathway of pyrimidine biosynthesis in the class Crustacea. Previously the pathway has been reported in the class Insecta (Porembska, Gorzkowski, and Jezewska, 1966; Moriuchi, Koga, Yamada, and Akune, 1972).

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Summary

1. The *in vitro* rates of incorporation of precursors into protein and RNA and the concentration of RNA were measured in tissues of intermolt and premolt lobsters acclimated to 5° C and 20° C. Midgut gland, abdominal muscle and gill of intermolt lobsters respond to temperature acclimation by a compensatory translation of the rate-temperature (R-T) curves with respect to the rates of incorporation of ^{3}H-leucine and ^{3}H-uridine into the acid-insoluble fraction. Midgut gland and muscle of premolt animals exhibit either no compensation or inverse compensation; gill tissue exhibits a rotation of the R-T curve.

2. The existence of the complete *de novo* pathway of pyrimidine biosynthesis is demonstrated in the class Crustacea. $NaH^{14}CO_3$ is incorporated into orotic acid and orotic-^{14}C-acid is incorporated into the acid-insoluble fraction.

3. Both the concentration of RNA and the rates of incorporation of precursors of both the salvage and *de novo* pyrimidine pathways are enhanced in the midgut gland of premolt lobsters, relative to intermolt tissue, under conditions of warm-acclimation.

LITERATURE CITED

Aiken, D. E., 1973. Proecdysis, setal development, and molt prediction in the American lobster (*Homarus americanus*). *J. Fish. Res. Board Can.*, **30**: 1337–1344.

Cole, W. H., 1941. A perfusing solution for the lobster (*Homarus*) heart and the effects of its constituent ions on the heart. *J. Gen. Physiol.*, **25**: 1–6.

Das, A. B., 1967. Biochemical changes in tissues of goldfish acclimated to high and low temperature—II. Synthesis of protein and RNA of subcellular fractions and tissue composition. *Comp. Biochem. Physiol.*, **21**: 469–485.

Das, A. B., and C. L. Prosser, 1967. Biochemical changes in tissues of goldfish acclimated to high and low temperature—I. Protein synthesis. *Comp. Biochem. Physiol.*, **21**: 449–467.

Drury, H. F., 1948. Identification and estimation of pentoses in the presence of glucose. *Arch. Biochem.*, **19**: 455–466.

Ennis, G. P., 1973. Food, feeding, and condition of lobsters, *Homarus americanus*, throughout the seasonal cycle in Bonavista Bay, Newfoundland. *J. Fish. Res. Board Can.*, **30**: 1905–1909.

Gorell, T. A. and L. I. Gilbert, 1971. Protein and RNA synthesis in the premolt crayfish, *Orconectes virilis*. *Z. Vergl. Physiol.*, **73**: 345–356.

Gwinn, J. F. and J. R. Stevenson, 1973a. Role of acetylglucosamine in chitin synthesis in crayfish. I. Correlation of ^{14}C-acetylglucosamine incorporated with stages of the molting cycle. *Comp. Biochem. Physiol.*, **45B**: 769–776.

Gwinn, J. F. and J. R. Stevenson, 1973b. Role of acetylglucosamine in chitin synthesis in crayfish. II. Enzymes in the epidermis for incorporation of acetylglucosamine into UDP-acetylglucosamine. *Comp. Biochem. Physiol.*, **45B**: 777–785.

Handschumacher, R. E. and C. A. Pasternak, 1958. Inhibition of orotidylic acid decarboxylase, a primary site of carcinostasis by 6-azauracil. *Biochim. Biophys. Acta*, **30**: 451–452.

Haschemeyer, A. E. V., 1968. Compensation of liver protein synthesis in temperature-acclimated toadfish, *Opsanus tau*. *Biol. Bull.*, **135**: 130–140.

HASCHEMEYER, A. E. V., 1969a. Studies on the control of protein synthesis in low temperature acclimation. *Comp. Biochem. Physiol.*, **28**: 535–552.

HASCHEMEYER, A. E. V., 1969b. Rates of polypeptide chain assembly in liver *in vivo*: Relation to the mechanism of temperature acclimation in *Opsanus tau*. *Proc. Nat. Acad. Sci. U.S.A.*, **62**: 128–135.

HAZEL, J. R. AND C. L. PROSSER, 1974. Molecular mechanisms of temperature compensation in poikilotherms. *Physiol. Rev.*, **54**: 620–677.

HOHNKE, L. AND B. T. SCHEER, 1970. Carbohydrate metabolism in Crustaceans. Pages 147–166 in M. Florkin and B. T. Scheer, Eds., *Chemical Zoology, V, A. Crustaceans*. Academic Press, New York.

HUGGINS, A. K. AND K. A. MUNDAY, 1968. Crustacean metabolism. *Adv. Comp. Physiol. Biochem.*, **3**: 271–378.

MCCARTHY, J. F., 1975. Temperature compensation in protein and RNA synthesis during the intermolt cycle of the American lobster, *Homarus americanus*. Ph.D. Thesis, University of Rhode Island, Kingston, Rhode Island, 140 pp. (*Diss. Abstr.*, **36B**: 610B; order no. 75–17,866.)

MCLEESE, D. W., 1956. Effects of temperature, salinity and oxygen on the survival of the American lobster. *J. Fish. Res. Board Can.*, **13**: 247–272.

MCWHINNIE, M., AND J. D. O'CONNOR, 1967. Metabolism and low temperature acclimation in the temperate crayfish, *Orconectes virlis*. *Comp. Biochem. Physiol.*, **20**: 131–145.

MORIUCHI, A., K. KOGA, J. YAMADA, AND S. AKUNE, 1972. DNA synthesis and activities of pyrimidine synthesizing enzymes in the silk gland of *Bombyx mori*. *J. Insect. Physiol.*, **18**: 1463–1476.

PASSANO, L. M., 1960. Molting and its control. Pages 473–536 in T. H. Waterman, Ed., *The physiology of Crustacea. I. Metabolism and growth*. Academic Press, New York.

POREMBSKA, A., B. GORZKOWSKI, AND M. JEZEWSKA, 1966. Utilization of 6-^{14}C orotate in the biosynthesis of pyrimidines in *Helix pomatia* and *Celerio euphorbiae*. *Acta Biochim. Polonica*, **13**: 107–111.

PROSSER, C. L. AND F. A. BROWN, 1962. *Comparative animal physiology*. W. B. Saunders Co., Philadelphia, Pennsylvania, 688 pp.

RAO, K. P., 1967. Biochemical correlates of temperature acclimation. Pages 227–244 in C. L. Prosser, Ed., *Molecular mechanisms of temperature adaptation*. AAAS Publ. No. 84, Washington, D. C.

SKINNER, D. M., 1965. Amino acid incorporation into protein during the molt cycle of the land crab, *Gecarcinus lateralis*. *J. Exp. Zool.*, **160**: 225–234.

SKINNER, D. M., 1966. Macromolecular changes associated with the growth of crustacean tissues. *Amer. Zool.*, **6**: 235–242.

SKINNER, D. M., 1968. Isolation and characterization of ribosomal ribonucleic acid from the crustacean, *Gecarcinus lateralis*. *J. Exp. Zool.*, **169**: 347–356.

SMITH, P. C., C. E. KNOTT, AND G. C. TREMBLAY, 1973. Detection of the feedback control of pyrimidine biosynthesis in slices of several rat tissues. *Biochem. Biophys. Res. Commun.*, **55**: 1141–1146.

VERNBERG, F. J., 1959. Studies on the physiological variation between tropical and temperate zone fiddler crabs of the genus *Uca*. III. The influence of temperature acclimation on oxygen consumption of whole organisms. *Biol. Bull.*, **117**: 582–593.

WEISS, H. M., 1970. The diet and feeding behavior of the lobster, *Homarus americanus* in Long Island Sound. *Ph.D. Thesis, University of Connecticut*, Storrs, Connecticut, 104 pp. (*Diss. Abstr.*, **31B**: 7245-B; order no. 71–16, 057.)

YAMAOKA, L. H., 1972. Protein synthesis in decapod crustaceans: The incorporation of ^{14}C-L-arginine into protein during the intermolt cycle in the dungeness crab, *Cancer magister*. *Ph.D. Thesis, University of Oregon*, Eugene, Oregon, 114 pp. (*Diss. Abstr.*, **33B**: 4481–B; order no. 73–7981.)

YAMAOKA, L. H. AND B. T. SCHEER, 1970. Chemistry of growth and development. Pages 321–342 in M. Florkin and B. T. Scheer, Eds., *Chemical zoology, V, A. Crustaceans*. Academic Press, New York.